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Tissue engineering of dermal blood and lymphatic microvascular networks

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14. ABSTRACT
This proposal focused on establishing the conditions necessary to induce lymphatic endothelial cell (EC) tube morphogenesis in 3D collagen matrices with the long-term goal of establishing separate networks of lymphatic tubes and co-existing, but not interconnecting networks of blood EC-lined tubes. In addition, we hoped that pericytes, which support blood EC tube networks, but not lymphatic vessel networks, would selectively recruit to one versus the other type of tube. Overall, we made progress, but did not identify the conditions that supported lymphatic tube morphogenesis over the long-term. We did identify some conditions that permitted early survival

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Report Title

Final Summary Darpa grant- DARPA proposal 07-21-Open-BAA-FP-135:
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Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

This proposal focused on establishing the conditions necessary to induce lymphatic endothelial cell (EC) tube morphogenesis in 3D collagen matrices with the long-term goal of establishing separate networks of lymphatic tubes and co-existing, but not interconnecting networks of blood EC-lined tubes. In addition, we hoped that pericytes, which support blood EC tube networks, but not lymphatic vessel networks, would selectively recruit to one versus the other type of tube. Overall, we made progress, but did not identify the conditions that supported lymphatic tube morphogenesis over the long-term. We did identify some conditions that permitted early survival and some tube formation, but generally this did not persist over the long-term and there were not extensive tube networks observed.

We did observe that pericyte addition to the lymphatic ECs were necessary for their survival in 3D collagen matrices under the conditions that we established. We also observed that the addition of sphingosine-1-phosphate and macrophage-CSF (M-CSF) appear to help lymphatic EC tube survival along with the pericytes. When tubes formed, often pericyte recruitment was observed, an interesting and unexpected observation. Because of these findings, we went back and analyzed whether our lymphatic EC populations, which were derived from human dermis, were in fact lymphatic ECs. We demonstrated that they express podoplanin, a lymphatic specific marker. Furthermore, when antibodies to podoplanin, were adsorbed to plastic surfaces, the lymphatic ECs attached to these antibodies, while a human blood EC (HUVECs) failed to attach. Previous studies in our laboratory have shown that the lymphatic ECs that we are growing do express Prox-1 and LYVE-1, which are additional markers that show considerable lymphatic EC specificity. Thus, we believe that the lymphatic ECs are expressing appropriate markers despite the confusing ability to recruit pericytes to some of the tubes that formed.

The final experiment that we performed did show some promise, where we began to substantially increase the density of lymphatic ECs in the cultures in addition to pericytes (compared to our normal lymphatic EC density). We wondered if there might be a factor produced by lymphatic ECs that might block pericyte recruitment, but it might be insufficient in amount unless sufficient lymphatic ECs were present. The result was suggestive that increasing lymphatic EC number may suppress pericyte recruitment relative to the lower density cultures. This is an interesting result that needs to be pursued further.

In conclusion, we have identified conditions which certainly improve the ability of lymphatic ECs to survive and form tubes in 3D collagen matrices. The overall survival over time is not optimal yet and thus, additional factors or other changes (such as altered cell densities or possible other missing cell types- e.g. macrophages?) are likely necessary in order for us to be able to engineer networks of lymphatic EC tubes. We were unsuccessful in our attempts to mix lymphatic and blood ECs together as they appeared to cross-inhibit each other. Thus, we spent our efforts in trying to establish the conditions for lymphatic cultures since we felt that this was absolutely necessary in order to eventually identify the conditions to successfully coculture both types of ECs in a 3D matrix environment.

Technology Transfer